

WHAT IS CLAIMED IS:

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1. A nucleic acid sequence for enhancing expression of a useful gene incorporated into a gene expression vector for enhancing expression of a useful gene comprising a nucleic acid sequence corresponding to a 5'-untranslated region of a viral gene or a fragment or a variant thereof.

2. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises at least one pyrimidine-rich tract.

3. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1 or 2, wherein the 5'-untranslated region comprises a sequence corresponding to a region selected from the group consisting of BoxA, BoxB, a trans factor-binding site, and a combination thereof.

4. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 3, wherein the 5'-untranslated region further comprises an AUG or ATG sequence.

5. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 4, wherein the 5'-untranslated region comprises a part of or an entire region of IRES of viral mRNA.

6. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 5 further comprises a portion of a coding region adjacent to the 5'-untranslated region, or a fragment or a variant thereof, of a viral gene in addition to said nucleic acid sequence.

7. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 6, wherein said nucleic acid sequence for enhancing

expression of a useful gene is incorporated downstream of an expression regulation promoter sequence and upstream of the useful gene in a gene expression vector.

8. The nucleic acid sequence for enhancing expression of a useful gene
5 according to any one of claims 1 to 7, wherein said nucleic acid is a cDNA sequence.

9. The nucleic acid sequence for enhancing expression of a useful gene
according to any one of claims 1 to 8, wherein said gene expression vector is a vector for
expression in eukaryotic cells.

10. The nucleic acid sequence for enhancing expression of a useful gene
according to any one of claims 1 to 9, wherein said virus is RNA virus.

11. The nucleic acid sequence for enhancing expression of a useful gene
15 according to claim 10, wherein said virus is picornavirus.

12. The nucleic acid sequence for enhancing expression of a useful gene
according to claims 10, wherein said virus is HCV virus.

13. The nucleic acid sequence for enhancing expression of a useful gene
20 according to claim 10, wherein said virus is HCV virus, and said nucleic acid sequence
for enhancing expression of a useful gene further comprises a portion of the coding
region for the core protein of the HCV virus or, a variant thereof.

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25 14. The nucleic acid sequence for enhancing expression of a useful gene
according to claim 12, wherein said nucleic acid sequence consists of the following
nucleotide sequence:

gccagccccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgtcgtgcag cctccaggac 120
30 cccctctccc gggagagcca tagtggtctg cggaaccggt gagtacaccg gaattgccag 180

(SEQ ID NO: 1, 1-180).

15. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of the following nucleotide sequence:

5 gacgaccggg tcctttcttg gatcaaccg ctcaatgcct ggagatttgg gcgtgcccc 60
gcgagactgc tagccgagta gtgttggtc gcgaaaggcc ttgtggtact gcctgatagg 120
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac c 161

(SEQ ID NO: 1, 181-341).

16. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of the following nucleotide sequence:

10 gccagcccc tgatggggg gacactccac catagatcac tccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggac 120
15 cccccctccc gggagagcca tagtggtctg cggaaccggt gactacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaaccg ctcaatgcct ggagatttgg gcgtgcccc 240
gcgagactgc tagccgagta gtgttggtc gcgaaaggcc ttgtggtact gcctgatagg 300
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac c 341

(SEQ ID NO: 1, 1-341).

17. The nucleic acid sequence for enhancing expression of a useful gene according to claim 13, wherein said nucleic acid sequence consists of the following nucleotide sequence:

20 gacgaccggg tcctttcttg gatcaaccg ctcaatgcct ggagatttgg gcgtgcccc 60
25 gcgagactgc tagccgagta gtgttggtc gcgaaaggcc ttgtggtact gcctgatagg 120
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac catgagcaca aatcctaaac 180
ctcaaagaaa aaccaaagct aacaccaacc gccgccaca ggacgtcaag tccccgggag 240
gtggtcagat cgttggtgga gtttacctgt tgccgcgcag gggccccagg ttgggtgtgc 300
gcgcgactag gaagacttcc gagcgtgcgc aacctcgtgg aaggcgacaa cctatcccca 360
30 aggtctcgcg gcccgagggc aggacctggg ctacagcccg gtatccttgg cccctctatg 420
gcaacgagg catgggggtgg gcaggatggc tcctgtcgcc ccgcggctcc cggcctagtt 480

ggggcccttc ggacccccgg cgtaggtcgc gtaatttggg taaggctatc gat 533

(SEQ ID NO: 1, 181-713).

18. The nucleic acid sequence for enhancing expression of a useful gene according to claim 13, wherein said nucleic acid sequence consists of the following nucleotide sequence:

gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggac 120
ccccctccc gggagagcca tagtggtctg cggaaccggt gactacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaaccgc ctcaatgcct ggagatttgg gcgtgcccc 240
gcgagactgc tagccgagta gtgttgggtc gcgaaaggcc ttgtggtact gcctgatagg 300
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac catgagcaca aatcctaaac 360
ctcaaagaaa aaccaaacgt aagaccaacc gccgcccaaca ggacgtcaag ttcccggggc 420
gtggtcagat cgttggtgga gtttacctgt tgccgcgcag gggccccagg ttgggtgtgc 480
gcgcgactag gaagacttcc gagcggtcgc aacctcgtgg aaggcgacaa cctatcccca 540
aggctcgccg gcccgagggc aggacctggg ctcagcccgg gtatccttgg cccctctatg 600
gcaacgaggg catggggttg gcaggatggc tctgtgcgc ccgcggctcc cggcctagtt 660
ggggcccttc ggacccccgg cgtaggtcgc gtaatttggg taaggctatc gat 713

(SEQ ID NO: 1, 1-713).

19. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 3 to 18, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence or a proximate sequence in at least one position corresponding to a pyrimidine-rich tract, BoxA, BoxB and/or trans factor-binding site contained in the 5'-untranslated region.

20. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 19, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion and/or addition of a single or a few nucleotides of

a sequence derived from a wild-type virus within the sequence corresponding to a region other than the 5'-untranslated region. *(D)*

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21. The nucleic acid sequence for enhancing expression of a useful gene according to claim 15, 16, 17 or 18, wherein said nucleic acid has one thymidine inserted into position 207 of SEQ ID NO: 1.

22. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 21, wherein said nucleic acid sequence for enhancing expression of a useful gene enhances expression of a useful gene by means of its own translation promoting activity.

23. The nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 22, wherein said nucleic acid sequence for enhancing expression of a useful gene enhances expression of a useful gene by means of accelerating IRES activity.

24. A nucleic acid sequence for enhancing expression of a useful gene comprising the following nucleotide sequence:

20 gccagccccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggcc 120
ccccctccc gggagagcca tagtggtctg cggaaccggt gactacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc 240
cgcgagactg ctaccgaggt agtggtgggt cgcgaaaggc cttgtggtac tgccatgatag 300
25 ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc 342

(SEQ ID NO: 7), which enhances expression of a useful gene by means of promoting mRNA translation in an IRES-dependent manner.

25. A nucleic acid sequence for enhancing expression of a useful gene which comprises a polynucleotide having a similar IRES activity to an IRES activity of the following nucleotide sequence:

gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggcc 120
ccccctccc gggagagcca tagtggtctg cggaaccggt gactacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc 240
5 cgcgagactg ctagccgagt agtggtgggt cgcgaaaggc cttgtggtac tgcctgatag 300
ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc 342

(SEQ ID NO: 7), and consisting of a fragment or a variant of the sequence, which enhances expression of a useful gene by means of promoting mRNA translation in an IRES-dependent manner.

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26. An isolated polynucleotide consisting of the following nucleotide sequence:

gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggcc 120
15 cccccctccc gggagagcca tagtggtctg cggaaccggt gactacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc 240
cgcgagactg ctagccgagt agtggtgggt cgcgaaaggc cttgtggtac tgcctgatag 300
ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc 342

(SEQ ID NO. 7).

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27. An isolated polynucleotide having a similar IRES activity to an IRES activity of the following nucleotide sequence:

gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggcc 120
25 cccccctccc gggagagcca tagtggtctg cggaaccggt gactacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc 240
cgcgagactg ctagccgagt agtggtgggt cgcgaaaggc cttgtggtac tgcctgatag 300
ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc 342

(SEQ ID NO: 7), and consisting of a fragment or a variant of said sequence.

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28. A gene expression vector comprising the nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 25.

Sub E1
28. 29. A host cell transformed or transfected with the vector according to claim

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30. A method of expressing a useful gene product using the vector according to claim 28.

31. A method for producing a useful gene product comprising the steps of:
growing the host cell according to claim 29 in a medium; and
10 isolating the useful gene product from the cell and/or the growth medium.

32. A method for enhancing expression of a useful gene product using the vector according to claim 28.

Sub D5
15 33. A probe for screening substances that interact with IRES, comprising the polynucleotide according to claim 26 or 27.

34. A probe for screening IRES-dependent translation initiators, comprising the polynucleotide according to claim 26 or 27.

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20 35. A therapeutic composition for treating diseases resulting from reduction of cap-dependent mRNA translation in a body of organisms, comprising the nucleic acid sequence for enhancing expression of a useful gene according to any one of claims 1 to 25 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.

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30 36. A therapeutic composition for treating diseases resulting from reduction of IRES activity in a body of organisms, comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 24 or 25 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.

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37. A method for determining the severity of hepatitis C, comprising the steps
of:

- 5 detecting the presence of a target polynucleotide sequence contained in a
biological sample derived from a test subject, by using the polynucleotide
according to claim 26 or 27 as the target; and
determining the severity of the hepatitis C based on the presence of the
sequence.

add a'7

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